THE EFFECT OF GAMBIER EXTRACTS (UNCARIA GAMBIR [ROXB.]) AS ANTISEPTIC ON GINGIVAL WOUND IN RATS

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antiseptic, bacteria, gambier

ABSTRACT

Background: The main principle in treatment of wounds is infection control by using antiseptic. Gambier (Uncaria gambir [Roxb.]) containing catechins and tannins reported that has an antiseptic effect. This aim of this study was to determine the effect of gambier extract as antiseptic on gingival wound in rats.
Methods: In vivo study, used pretest-posttest only control group design had been conducted in Animal house, Medical Faculty of Sriwijaya University and Province's Health Laboratory in Palembang. There were 30 white Wistar rats divided into five groups. Group 1 was given 10% povidone-iodine ointment (positive control), group 2 was given a placebo ointment (negative control), group 3, 4, and 5 were given an ointment with 10%, 15%, and 20% gambier extracts. Gingival labial wound of the mandible was induced with cylinder diamond bur, then swabbed before and after treatment. Gingival swab samples were cultured in agar medium and incubated for 24 hours. The number of bacterial colonies from all groups were counted by colony counter. The statistical analysis was used IBM SPSS statistics version 22,0.
Result: The result showed that the number of bacterial colonies from all groups decreased significantly after treatment, except for negative control. The higher concentration of gambier extract led the better effect of antiseptic.
Conclusion: It can be concluded that gambier extract has antiseptic effect of gingival wound in rats.

INTRODUCTION

Wounds in the oral cavity happen due to brushing teeth too strong, extraction of teeth, biopsies, scaling, and inappropriate preparation of the teeth[1,2,3]. The wounds that are not treated properly will lead to loss of all or part of the function of organs, bleeding, cell death, and bacterial contamination. Contamination of bacteria will cause an infection in the wound[4]. The main principle in treatment of wounds is infection control by using antiseptic[1,5]. The antiseptic most widely used is 10% povidone-iodine. Some studies show that 10% povidone-iodine effective as a topical antimicrobial in the prevention of caries in early childhood[6,7].

Since ancient time, Indonesian people practiced traditional medicine. One of them is gambier (Uncaria gambir [Roxb.])[8]. Gambier is used traditionally for various purpose, such as to treat diarrhea, influenza, dysentery, stomatitis, cough, sore throat, and gingivitis [9,10]. The earlier studies of this plant demonstrated that it had antioxidant property, potent anti-inflammatory, anti bacterial [11,12]. Rosada et al. reported that 24 mg gambier extract was effective in decreasing bacterial colonies on male Wistar rats [13]. Merta et al. reported that ethyl acetate gambier extracts inhibited the growth of Staphylococcus aureus [14]. Pambayun et al. stated that 22.26% gambier extract had antibacterial properties against bacteria gram-positive Streptococcus mutans, Bacilus subtilis and Staphylococcus aureus,

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respectively [15]. Katu et al. showed that 1% concentration of gambier extract and contact time of 24 hours effectively inhibited the growth of Enterococccus faecalis [16]. The antimicrobial activity of gambier comes from the main contents, such as catechins and tannin [17]. Catechin is complex flavonoid compound from polyphenol group, that has antioxidant and antibacteria properties [18]. Antimicrobial activity of catechin is due to its ability to damage cell membrane and bind to ATP site of DNA gyrase b subunit [19]. Magdalena et al. reported that gambier was more effective in inhibiting Gram positive bacteria rather than Gram negative bacteria [20]. Catechin derived from gambier penetrates easily to peptidoglycan, disrupts cell wall structures and functionality, and leads to cell lysis [21]. There are millions of Gram positive and Gram bacteria can be found in oral cavity, that can cause infection due to the opened wound. The major causative bacteria associated with wound infection are Staphylococcus aureus, Streptococcus pyogenes, and Pseudomonas aeruginosa[22,23].

Gambier extract compounds suspected to have antiseptic effect on wound, because some bacteria were highly sensitive to catechin contained in gambier. In this study, we investigated the effect of gambier extracts (Uncaria gambir [Roxb.]) as antiseptic on gingival wound in rats.

METHODS

The study was conducted in Animal House of Medical Faculty, University of Sriwijaya, Palembang, Indonesia and Province's Health Laboratory of South Sumatera, Palembang, Indonesia. The protocol had been approved by Health Research Review Committee, Mohammad Hoesin General Hospital and Faculty of Medicine Sriwijaya University, with the certificate of ethics number: 469/kepkrsmhfkunsri/2017.

Subjects of this study were 30 male Wistar rats (Rattus norvegicus), aged 10-12 weeks, weight 150-250 grams and in good health. Wistar rats suffered from physical disabilities and comorbidities were excluded. Rats were obtained from Bandung, West Java, Indonesia, with the animal health certificate from Departement of Agriculture and Food Security no. 524.0/1755-Dispertapa/2017. Subjects were divided into five groups. Group 1 was given 10% povidone-iodine ointment (positive control, Betadine oint®), group 2 was given a placebo ointment (negative control), group 3, 4, and 5 were given an ointment with 10%, 15%, and 20% gambier extract concentration.

Animal preparation

Preparation starts with acclimatization. Acclimatization is the process of adjusting the experimental animals from climate environment change. Samples were acclimatized in a room for a week at room temperature of 200-250 for 8 days. At the time of acclimatization, rats were fed with pellets for 20% from rats body weight and 45 ml/day water. Samples were placed in a 5 square enclosure made of plastic and wire. Each cage is partitioned into 6 section to fill 1 rat in each section.

Gambier extracts

Gambier was taken from Babat Toman, Sekayu, Indonesia and had been identified and authenticated by Faculty of Agriculture of University of Sriwijaya. Gambier preparations size 40-60 mesh was weighed as much as 60 grams, wrapped in filter paper and inserted in the tube soxhletation (PyrexTM, USA). Soxhletation was filled with ethyl acetate 98% up to 300 ml followed by heating at a temperature of 77 ° C, allowed to circulate until

the solvent becomes clear for 7 hours. After that, an evaporator (EBE-1, Oxford Sci. Ltd., USA) was used to evaporate the whole solvent for 2 days to obtain 100% dry gambier extract.

Gambir extract ointment

The dose conversion of ointment used in this study was 50 mg for each rat so that the amount of ointment needed within a day used by 6 rats was 300 mg[24]. Ointment base was the basis of fat by comparison according to the standard formula ointment base that is 15% adeps lanae and 85% vaseline album[25,26].

a. Negative control group:
   R / Adeps lanae 45 mg
   Vaseline album 255 mg
   m.f placebo ointment 300 mg
b. Gambier extract groups:
   a) 10% Gambier extract
      30 mg Gambir extract was mixed with 40.5 mg and 229.5 mg adeps lanae vaseline album to be 300 mg.
   b) 15% Gambier extract
      45 mg Gambier extract was mixed with 38.25 mg adeps lanae and 216.75 mg vaseline album to be 300 mg.
   c) 20% Gambier extract
      60 mg Gambir extract was mixed with 36 mg and 204 mg adeps lanae vaseline album to be 300 mg.

Firstly, mortar was heated in the oven at 50 degree Celcius for 10 minutes, then hot mortar was removed from the oven. Secondly, adeps lanae was inserted into mortar, stirred with the pestle, mixed with vaseline album, stirred at constant speed until homogeneous. This ointment base was placebo and used as negative control. For group 3,4,5, gambier extract was added according to the concentration of each group and stirred until homogeneous. All the ointments were put into the labeled containers.

Gingival wound model

All rats were anesthetized using ketamine hydrochloride (50 mg/kg, i,p., body weight). Labial gingival of rats was cleaned with sterile wetted cotton. Area of the wound to be created was marked. The lower lip was pulled using tweezers and gingival labial wound was induced by cylinder diamond bur. Blood was cleaned. Samples were taken with sterile cotton swab twice with alternating movements. After that, placebo (the ointment base), standard drug (10% povidone-iodine, Betadine oint®), formulated gambier extract ointments were applied for a minute and wound was swabbed by using sterile cotton swab twice with alternating movements.

Counting Bacterial Colonies

Samples, taken by cotton swab, were inserted into 10 mL test tubes filled with 1 mL Phosphate Buffered Saline (PBS), homogenized and closed with sterile cotton, then put into ice box. Samples were sent to Province's Health Laboratory of South Sumatera, Palembang.

Samples were cultured aseptically with two flame bunsen burners. Bacteria in the mixture were diluted one fold by adding 9 mL PBS. The tubes were rotated for 30 seconds to get homogeneous mixture. One mL liquid from the mixture was taken from the tubes and put into sterile petri dish and labeled each sample according to the groups. Plate Count Agar (PCA) was poured as much as 25 cc, homogenized by shaking the petri dish, allowed to solidify, incubated at 35-370C for 24 h in reserve position under static condition. The number of bacterial colonies from all group was counted by colony counter (SC6 Plus, Stuart ®., UK).

Petri dish was placed on the electronic pressure pad of colony counter. A transmission

light array with magnifier was used to count the number of colonies. Counted CFU was marked with a felt tip pen on the plate cover to discriminate counted from uncounted colonies or to avoid double counting. Touch pressure caused a count to be registered on the digital display and an audible tone confirms each count made. The sensitivity of the electronic pressure pad is adjustable to suit the user. The examining data were recorded.

Statistical Analysis

Statistical analysis was performed using SPSS 22 vs (IBM® Inc.pvt ltd.) and Microsoft Excel (Microsoft Inc®). Data were recorded and written in the form, then processed, and analyzed. Before further processing, data were tested with Saphiro Wilk normality test to find out the normal distribution of data and Levene's test to know the homogeneity of samples. If p> 0.05, meant data were normal and homogenous . Extended Paired t-test was used to compare the changes between "before and after" experiments. Independent t-test was examined to compare the efficacy between groups in this study. One way Anova was used for the significance of difference in all groups. To know the compatibility dose, Post Hoc test was used. Level of significance for all hypotheses was 5%.

RESULT

Based on the normality test and Levene's test, the data from all groups were normally distributed (p>0.05) and homogenous (p>0.05). For the next step, we performed paired t-test

Table 1. The effect of the number of bacteria colonies within groups

Group	The number of bacterial colonies		P value
	Pretest	Posttest	
	Mean±SD		
Positive control (K1)	89.67±05.89	25.17±06.31	0.00*
Negative control (K2)	81.00±11.22	119.67±11.02	0.00*
10% gambier extract (K3)	89.50±13.78	79.17±07.55	0.00*
15% gambier extract (K4)	85.67±06.09	62.67±05.01	0.00*
20% gambier extract (K5)	89.83±11.75	47.67±10.31	0.00*

Paired t-test, p=0.05 *significance

Table. 2 The effect of the number of bacteria colonies between groups

Groups	Groups	p value
Positive control	Negative control	0.00*
	10% Gambier extract	0.00*
	15% Gambier extract	0.00*
	20% Gambier extract	0.00*
Negative control	10% Gambier extract	0.00*
	15% Gambier extract	0.00*
	20% Gambier extract	0.00*
10% Gambier extract	15% Gambier extract	0.00*
	20% Gambier extract	0.00*
15% Gambier extract	20% Gambier extract	0.01*

Independent t-test. p=0.05 *Significance

Table 3. Compatibility dose in reducing bacterial colonies

Variable	Positive Control	Negatif control	10% gambier Extract	15% gambier Extract	20% gambier extract
Positive control		0.00*	0.00*	0.00*	0.00*
Negatif control	0.00*		0.00*	0.00*	0.00*
10% gambier extract	0.00*	0.00*		0.00*	0.00*
15% gambier extract	0.00*	0.00*	0.00*		0.00*
20% gambier extract	0.00*	0.00*	0.00*	0.00*	

Post Hoc LSD. p=0.05 * Significance

to compare the efficacy of gambier extract on gingival wound within groups. The results were illustrated in table 1.

From table 1, it showed that all groups had ability in reducing bacterial colonies significantly, before and after giving treatment, except negative control. The highest number of decreasing bacterial colonies was exhibited in positive control group, followed by 20%,15% and 10% gambier extract.

Table 2 showed that there were significant differences between groups after treatment. It meant that all gambir extracts in 10%.15%. and 20% concentration could eliminate the number of bacterial colonies compared to placebo. Povidon iodin presented the best effect of reducing the number of bacterial colonies. Data analysis was continued to one way ANOVA test and the result obtained from the test was p value=0.00. It meant that there was significant difference among all groups in declining bacterial colonies. Post hoc LSD test was performed to identify the compatibility dose of various concentrations of gambier extracts in affecting the number of bacterial colonies.

Table 3 described that all groups of gambier extract reduced bacterial colonies compared to placebo ($p<0.05$), but none of the gambier extract groups had the same effect as the positive control group.

DISCUSSION

This study revealed that the number of bacterial colonies decreased significantly after giving gambier extract ointment, so it proved that gambier has antiseptic effect. Voravuthikunchai et al demonstrated that Uncaria gambir had ability in inhibiting and killing Helicobacter pylori[27]. Melia et al. evaluated that gambier extract had antimicrobial activity in Gram-Positive bacteria (Staphylococcus aureus) and Gram-Negative bacteria (E. Coli and Salmonella sp.)[28]. Dewi et al. found that gambier extract had anticariognic effect due to its ability in reducing Streptococcus mutans colonies in artificial saliva, declining micropore on enamel, and lowering the decline of calcium weight in teeth[29].

Antimicrobial activity of gambier is due to the presence of high amount of total phenolics and flavonoid[30]. Kassim et al. reported that ethyl acetate gambier extract contained total phenolic content 113.43 mg/g extract, total condensed tannin determination 93.12% wt, total flavonoids content 93.31 mg CE and catechin contents 87.33% wt[31].

Phenolic compounds are the main antibacterial agents. Phenolic compounds

interact with membrane proteins, change cell permeability, destroy membrane structure and functionality. Increased membrane permeability causes a loss of cellular integrity and results cell death[32]. Previous study also reported that phenolic compound was related to inactivation of cellular enzyme and further lead to loss of cell viability[33].

Tannins contained in gambier are polymeric substance, that have ability as antimicrobial agents. Condensed tannins inhibit extracellular enzyme of bacteria, induce complexation of bacterial substrates, and disrupt their metabolism through interference of oxidative phosphoylation[34,35]. Dos et al. demonstrated that tannins isolated from Solanum trilobatum Linn. possed toxic activity against bacteria. This activity was related to their actions on the microorganism membrane[36]. Condensed tannins are capable of binding to cell walls, inactivating microbial adhesins, inhibiting cell envelope transport proteins, inducing bacterial statis and protease activity[33,35]. Tomiyama et al. indicated that condensed tannin extracted from Astringent persimmon (PS-M) contained in refreshing beverages commercially in Japan had antibacterial effects against polymicrobial biofilms. The effectivity was equal to chlorhexidine[37]. Tannins also can neutralize bacterial toxin. Choi et al. stated that apple condensed tannin resisted alpha-toxin cytotoxicity of Staphylococcus, so the pathogenesis of Staphylococcal infection decrease[38].

Flavonoids are group of polyphenols synthesized by plants that have many benzene rings in their structure. Godstime et al. reviewed that flavonoids exhibited antimicrobial response against Vibrio cholerae, Streptococcus mutans, Shigella, and some viruses[34]. Flavonoids have several mechanisms in suppressing pathogens. Flavonoids inactivate bacterial adhesion and cell envelope transport proteins, agitate cell membranes, change membrane permeability, disrupt the respiratory chain. Flavonoids intercalate hydrogen bonds with nucleic acid bases and cause inhibition of DNA and RNA synthesis[39]. Wu et al. reported that hydroxyl group substitution of flavonoid inhibited DNA gyrase from E. Coli. The inhibition of DNA gyrase might trigger programmed cell death and lysis[40].

Another study presented that antibacterial activity from flavonoid was coming from the cytoplasmic membrane damage due to perforations, inhibition of a membrane-embedded enzyme, and reduction of membrane fluidity by generating hydrogen peroxide[41]. Flavonoid binds to cell surface, penetrates to the target sites, inhibits the respiratory chain components, suppresses oxidative phosphorylation and active transport, disturb DNA, RNA, protein and lipid synthesis[42]. Krishnan et al. demonstrated that Marchantia linearis flavonoid treated in B. cereus, B. subtilis, S. aureus, and S. typhimurium accelerated leakage from cytoplasm and damaged cell membrane[43]. The leakage of cytoplasmic membrane induces the loss of nucleic acids and proteins[41,43].

Gambier also has large amounts of polyphenol constituent in the form of catechin.[16] Andarsuryani et al. demonstrated that catechin content in gambier could be determined by using NIR spectroscopy[44]. Catechin is polyphenol that has antimicrobial activities. Those activities are due to its interaction between catechin and lipid bilayer. Catechin decreases the membrane fluidity in both hydrophilic and hydrophobic of lipid bilayer and causes loss of membrane structure, then leads to cell death[45]. Gopal et al. reported that catechin was an

antibacterial activity against dental bacterial in human, such as Streptococcus mutans[19].

Povidone-iodine shows the best result in decreasing bacterial colonies on wound. This condition is due to its ability to react with phenolic groups, such as –OH and amino acid, such as –NH2, so that iodine can penetrate to bacterial biofilm and disturb metabolic processes of microbial[46]. Povidone is effective in killing Gram negative and Gram positive bacteria. Kanagalingam reviewed that the antibacterial effect of povidone-iodine was faster and stronger than cetylpyridinium and hexitidine[47].

Gambier extract is effective as antiseptic on mucosal wound in a dose-dependent manner. It might have the same effect as povidone-iodine if the concentration of ethyl acetate gambier extract is improved.

CONCLUSION

Ethyl acetate gambier extract (Uncaria gambir) has antiseptic effect on gingival wound of white rats wistar strain in a dose-dependant manner.

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